MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

DATE:	February 29, 1996
SUBJECT:	Review of Product Sections in PLA 95-1167
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TO:	File
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This is a review of product sections and associated information assigned by the PLA committee chair.

B.3 Storage of Stock Cultures and Seed Lot System

A description of the preparation and storage of the MCB and MWCB, for cells containing the — (production), or — (development) promoter is described. For cells containing the — promoter, a MCB consisting of — vials was created on 1.12.1992 from — containing the sequence encoding reteplase. A MWCB of — vials was prepared from — the MCB on 9.12.1992. The freezing media contains — glycerol (final concentration). The MCB and MWCB are housed in the gas phase of liquid nitrogen freezers dedicated to the storage of cell banks that are located in separate buildings. For cells containing the promoter, a MCB of — vials was made on 7.4.1989 and a MWCB of — vials on 25.4.1989 from a — containing the sequence encoding reteplase. The glycerol concentration used in these preparations is — (final concentration). No location is indicated for storage of these MCB or MWCB.

Reasonable care was described in the procedure for establishment of the cell banks. This included using a dedicated laboratory, — for selection, and identity testing on the resulting banks. SOPs are referenced, but not provided. There are sufficient numbers of vials in the — promotor-containing MCB and MWCB from which to isolate adequate product over the expected lifetime of reteplase. This conclusion is based on the manufacturing scheme where one vial is expanded into a — fermenter from which a single batch of inclusion bodies (IB) are isolated. Up to —, are processed to form one lot of bulk reteplase. Several bulks depending upon the amount of material are combined and formulated to yield approximately — This would treat — patients following the standard 10 U + 10 U regiment.

C.1 Renaturation and Purification

Renaturation

Renaturation occurs in an area and with equipment dedicated to the production of reteplase. The renaturation process is briefly summarized. The isolated C

protein into various configurations including the active structure for reteplase.]

The ——— is renatured using the technique of ——————————. period in an aqueous buffer containing ——————— to support refolding to the native and hence active reteplase conformation. Pulsed renaturation is the sequential additon of mixed (——— at fixed time intervals. This sequential addition results in a low concentration of denatured proteins at any given time. Since renatured proteins are less likely to aggregate than denatured proteins, this process favors protein renaturation and mimimizes protein aggregation.

The manufacturer indicates that the folding process has been validated and the results from several production runs confirm that this complex process is well-controlled and reproducible. The performance validation reports (section C.9.c), two studies that subjected refolding to stress conditions (see below - capacity of chromatography media), and in-process controls from the commercial production runs do indicate that the folding process is consistant. The reported average yield of authentic (correctly folded) reteplase after renaturation is ——— From a viewpoint of production consistency and quality of the active ingredient, this is reasonable for this process considering the possible permutations and potential for incorrectly folded molecules from denatured reteplase that contains ———.

A critical manufacturing component during the renaturation procedure is the C] Buffers used in chromatography are also sparged, but a validation study indicates that oxygen does not affect purification yield or activity. Sparging with an inert gas reduces the oxygen content leading to conditions that favor an improved efficiency of recovering active product (Vol 4, pg 853). There appear to be no limits established for the oxygen or nitrogen content in vessels. Since the sponsor has demonstrated that this inert atmosphere is critical for product yield, appropriate limits should be established and set. Alternatively, the SOPs should be written and validated to ensure that upon execution of the procedure a consistent atmosphere would be achieved. Appropriate documentation should be in the batch record to ensure that this process is consistently performed.

Purification

Active reteplase is purified using C]

chromatography. The purification process relies exclusively on chromatography to bind the active site of correctly folded reteplase separating them from other incorrectly folded variants. , it must be tightly controlled. Cation exchange chromatography is performed on a column that removes protein and non-protein impurities and results in a reteplase purity of >98%. This chromatography step removes process-induced impurities - dimers and oxidized and molecules. Lastly, chromatography removes trace impurities such as DNA and endotoxins.

The general purification scheme is summarized. Purification is performed in a dedicated suite with dedicated column materials. The renatured solution is

results are presented for several IPC from batches manufactured during scale-up of various steps in the manufacturing process. Noticable changes during the scale-up have been attributed to changes in the process. My review of the data indicate that, overall, the process produces material with consistant activity, and yield that is proportional to the starting material.

Column regeneration protocols, and short term and long term storage conditions are described (see below). Representative chromatograms are presented for each column. All product pools are tested for compliance to the IPC limits and meet these limits (see below).

In-Process Controls

For manufacture of the bulk, in-process controls (IPC) are performed on samples taken at the following process steps: solubilization, modification, renaturation, filtration, all chromatography steps, dialysate, and bulk drug. The parameters measured for renaturation are The parameters for purification steps are

After final filtration, the bulk is assayed

for specific activity, sterility (21 CFR 610.12), and dimer content.

Appropriate parameters are in place for each manufacturing step. The listed parameters have sufficient limits to indicate that the reduction [redacted], modification [redacted], renaturation (activity), and protein refolding (specific activity) is functioning at various process steps. Upper protein concentration or activity limits, obtained from validation studies, have been established to prevent overloading of the column (see Capacity of Chromatography Media). For most in process parameters, limits were based upon the average +/- 3 Standard Deviations from [redacted] commercial scale runs (see Report B.3.2.5, Vol7. Pg 1432) Consequences ranging from investigation of cause to rejecting material are listed if limits are not met.

However for the purification process, no rationale is provided for setting the limits for bioburden and endotoxin parameters. If the measured bioburden is [redacted]

established/revised to reflect the actual manufacturing levels with consequences of investigation and disposal, respectively. Bioburden and endotoxin are sensitive indicators that the process is well controlled and is often an early warning that a problem has developed.

Regardless of the amount of protein isolated from the [redacted] columns, they are not discarded due to "monetary considerations". While results from purification at the commercial scale indicate that there have been reasonably high yields from these columns, what is the minimal amount of product that would be collected? Would as little as one gram or less be collected? Potential problems with collecting small amounts of protein could include a chromatographic failure, or increased amounts of contaminants. Presumably an unexpected reduced amount of material from a chromatographic failure would be treated as reprocessed material (see below - reprocessing). At low protein levels does the ratio of contaminants to the amount of protein increase? Data from the validation studies indicate that the column capacity should not be exceeded, and therefore should not result in an increase in contaminants. Should a lower limit be set on the amount of product collected from these columns or should any amount be collected?

General Process

The hold times for drug intermediates used in the manufacturing process are determined in the validation studies (see below - Stability of Process Intermediates). [redacted]

The reprocessing criteria are described as:

These criteria are stringent and well defined. Only one reprocessing step is allowed during the purification process, starting with chromatography step. Reprocessing of material prior to this step is not allowed. Reprocessing is supported by validation studies demonstrating that repeating any one chromatographic step doesn't affect the bulk substance specifications. Deterioration of IPC does not occur until the third repeat. Reprocessing is allowed after filling of bulk drug if it doesn't meet sterility limits. No information is provided on the control, evaluation, or release of reprocessed product. The appropriate SOP that describes this information should be provided.

C.2.A. & B Raw Materials for Fermentation, Harvest, IB-Isolation, Renaturation and Purification.

Some materials are of pharmacopoeial quality, although materials of varying quality are also provided. There are no materials of animal or human origin, nor are any components listed as derived from animal or human origin.

Specification sheets that describe product specifications with an acceptable limit, value, or range for various parameters are included for each material. Confirmatory testing is performed at Boehringer Mannheim (BM) for some materials as indicated in the ELA, and by reference test methods numbers on some specification sheets. Most specifications sheets contain parameters with specifications for characteristics, identity, and purity (including contaminant levels). Although no test methods are provided, many reference compendial tests (USP, DAB (German equivalent)). Appropriate parameters are measured for the components especially critical components such as . Minimal storage conditions, stability times and special instructions are provided for most materials.

Regarding certificates of analysis required and identity tests performed on all materials or as per 21 CFR 211.84.(d)., in the ELA, it is indicated that most raw materials are tested at BM to meet established specifications. A few materials (filters,) are accepted based on certificates of analysis and identity tests. Gases are accepted solely on certificates of analysis. The ELA also describes the sampling procedure used for testing.

The manufacturer's identity is not provided, with the exception of those materials indirectly identified by submitted certificates of analysis (filters and inert gas). There is no information on the vendor qualification program.

C.3. Cleaning, Regeneration and Sterilization Systems
Each chromatographic column is regenerated after a chromatography cycle. According to an abbreviated description of the process, C

a fixed incubation time should be specified so as to exert a constant effect on the column performance (see below).

Regeneration procedures for all columns were tested in scaled down studies using resin from a production column after an unspecified number of chromatographic cycles. The material was split, a mock purification performed on some samples, and analyzed for impurities - C

The tabulated results indicated that proteins present on all columns before regeneration, did not remain bound or were eluted after treatment.

From the data presented, the procedures are effective in removing protein contamination. It would be useful to know the number of production cycles that each chromatography resin been exposed to in order to ensure that the regeneration procedure is still as effective near the end of the column lifespan.

Column Storage
C

This should provide adequate measures to ensure the sterility of the column.

Column Lifetimes
It is indicated that the column resins are dedicated to the manufacture of reteplase and are reused for multiple chromatographic cycles. The maximum cycle number is set for each column resin - C

The stability and maximum number of production cycles of

<] was calculated from the stability data of the suppliers, while that for [ ] column was calculated from a scaled-down validation experiment. It is also indicated (Vol 4, pg 711) that after the maxium cycles are reached, a further evaluation is performed and if the performance specifications are met and do not reach IPC parameters for all batches the column can be released for further use.

For [ ], the stability and maximum number of cycles was determined in separate scaled-down studies following the chromatographic and regeneration procedures used in production with some changes - notably the validation column [ ] regeneration. In the validation study, there is a loss of protein and total activity such that at [ ] The quality of product from the validation procedure as measured by specific activity and dimer content is not affected. The impurity profile of reteplase is reported not to change based upon data from several commercial scale runs.

BM states that this yield decrease is not attributable to column fouling, but is dependent on the decreased binding capacity of the [ ] BM calculated time of [ ] time the ——— in production were treated with ——— compared to the total time the validation column was incubated in ———

The calculated ——— lifespan is only an estimate that is based on assumptions regarding the denaturating effects of ——— over time that is unsubstantiated by data or reports. The column lifespan for this major purification procedure should be based on a validation study that accurately reflects the commercial scale conditions which this study does not, or on real time, real condition data preferably at a commercial scale. Additional support can be obtained by providing information on the submitted pilot and commercial scale ——— column runs, if additional information including the regeneration ——— times are provided. This information should be readily available from the sponsor. In addition, if the regeneration procedure with ——— is time critical as to effect the [ ]

BM cites data from the chromatography suppliers that no effect is seen on the chromatographic properties of the [ ] their regeneration procedure. BM calculated the maximum number of production cycles from stability data and the incubation time during the stability data to arrive at ——— This column lifespan should be supported by real time, real condition data or a validation experiment. Some data can be obtained from the ——— columns used in pilot and commercial scale if the appropriate use information is provided.

Capacity of the Chromatography Media

The capacities of the chromatography media were used to define the protein loads and load volumes for the production columns used for purification. For [redacted] the capacity of the column was based upon the binding activity of the resin allowing for decrease in capacity over time. Column volumes were calculated from the protein load (protein activity/capacity release limit). Results from the column lifetime study indicate that overloading the column will not affect quality of the product. For the [redacted] were used to determine the load. Alert and action limits based on the [redacted] content were determined. Since impurities bind to the [redacted] column, the load was based on the maximum content of impurities in the load. This value of [redacted] of gel is far below the [redacted] binding capacity of the column. The data and results presented support the limits. Appropriate [redacted] are present in the manufacturing process that should detect problems if these values are exceeded.

A report describing the removal of incorrectly folded reteplase molecules by [redacted] chromatography was reviewed. The validation study subjected the refolding process to stress conditions that including [redacted] reteplase as indicated by higher amidolytic activity and a decrease in the enhancement of plasminogenolytic factors. [redacted] chromatography removed all of the altered molecules as indicated by HPLC analysis. However, the [redacted] form may still remain, and so is analyzed for in the bulk and final product.

In a second study, samples were exposed to [redacted] the manufacturing process [redacted] or chromatography step in the purification process. Data indicated that [redacted]

BM indicated that [redacted] purification had no impact on final product quality. The data presented supports this conclusion.

C.5. Storage of Bulk Drug Substrate

The bulk drug is stored in an adequately labeled, [redacted] at [redacted] varehouse. They are shipped [redacted] to the Mannheim facility. There is conflicting information in the PLA as to packaging of the bottles for shipment. No validation of the shipping conditions are in the PLA or ELA. Additional details/SOP for shipping, and the validation of the shipping procedure should be provided (see Preparation of Final Bulk). By agreement with the ELA chair these issues will be raised in the ELA review..

C.6. Stability Data

A. Manufacturing Date of Bulk

BM defines the manufacturing date or date of production of the bulk as when the lot specific data is logged into the electronic tracking system, and the batch is transferred from the production department to the warehouse.

B. Stability of Process Intermediates

Storage of intermediates of the production process have defined time and temperature as determined by validation studies.

Stability of Process Intermediates

Intermediate	Batch(s)	Tests	Storage Temperature	Storage Time
[redacted]	D007	Protein content % Protein	[redacted]	[redacted]
Crude Inclusion Bodies	D007	Protein content % Protein	[redacted]	[redacted]
Purified Inclusion Bodies	D002 D010 D011	Protein content % Protein Total Protein Total Activity Amidolytic Activity Dimer Content	[redacted]	[redacted]
Mixed Disulfides	G005 (pilot) G012 G013	Protein Conc. SH Content Turbidity Total Protein Total Activity Specific Activity Dimer Content	[redacted]	[redacted]
[redacted]	G012 G013	Total Yield Spec. Activity Dimer Content Bioburden Endotoxin	[redacted]	[redacted]

Samples were taken from production or pilot batches, analyzed for stability indicating parameters, divided into aliquot and stored at various temperatures. Prior to measuring protein stability indicating parameters, some intermediates were further manipulated following the manufacturing process at a reduced scale. This includes denaturation, modification, renaturation, and purification using column chromatography.

All stability tests were conducted for periods that exceed the stated storage times, unlike hold times for several intermediates in the formulation and manufacture of the drug substance (see below - commercial scale process validation). Considering the variability inherent in the stability indicating assays, the data substantiates the stated storage claims, even though no statistical analysis was reported.

Furthermore, the indicated storage times are all exceeded by testing of intermediates stored beyond the claimed time limit.

However, the biomass and crude inclusion bodies times were tested only one time and from one lot. Therefore, stability studies should be repeated on at least another lot of material for the biomass and crude inclusion bodies. Although a hr time point is claimed for mixed disulfides stored at , data is present for an day time point on two lots. Data from samples at s, indicates that the specific activity is close to the lower acceptable limit. An accurate assessment of intermediate storage times cannot be made since no specific activity data is present for intermediate time points. This holding time for mixed disulfides may be important since they are added over approximately days, and the holding conditions are unknown.

No description of the storage containers for each intermediate are provided. On inspection please check to see that the storage containers for each intermediate are appropriate and specified in the SOP(s).

B. Stability of Bulk Drug Substance

Two studies are cited in support of dating for the Bulk Drug Substance. Study 1 is conducted with bulk lots made from cells with the - two pilot , and one commercial)(see table). Study 2 is conducted with bulk lots made from cells containing the on a commercial ( )scale.

Table. Overview on the Stability Program

(Status as of April 1995)

Study No.	Batch	Scale/ Promotor	Date of Production/ Release	Concentration[1)] [mg/mL]	Stage [month]	Duration [month]
1	G001.00		Dec. 2, 91			
	G002.00		Dec. 18, 91			
	G007.00		March 19, 93			
2	G010.00		Dec. 21, 93			
	G011.00		Dec. 21, 93			
	G012.00		June 30, 94			

1) C2 and C3 are concentrated forms of the bulk drug substance (C1).

The bulk stability samples in [redacted] buffer are stored in [redacted] containers - the same container material that the bulk is stored in. The samples are stored at [redacted]. The lots are tested every [redacted] months in the first year and every [redacted] months thereafter. Different parameters were used in each study to measure the bulk stability. The parameters analyzed in Study 1 were [redacted] and absorbance at [redacted]

The parameters and specifications used in Study 2 are indicated below.

Table 4: Test parameters and specifications used in study 2

Parameters	Specifications in Stability Study
Amidolytic activity	[redacted]
Clot-lysis activity	[redacted]
Apparent molecular weight and degradation products	[redacted]
Detection of degradation products	[redacted]
Two-chain (tc) form	[redacted]
Modifications	[redacted]
Dimers and Aggregates	[redacted]
Appearance of solution (absorbance at [redacted] nm)	Report results [1]
Degradation, modifications (peptide map)	Pattern corresponds to working standard

[1] No significant change over time

The fibrinolytic activity was used as a potency assay prior to the development of the clot lysis assay as described for the reteplase activity standard. The [redacted] was also changed for Study 2 in order to detect the two-chain form and modified species. All assays are performed with each stability determination except for peptide mapping that is performed every [redacted] months. Test methods are described in PLA Item C.7. Future stability studies using test parameters and specifications listed in Study 2, without the MW and peptide mapping analysis, will be conducted periodically on new batches.

BM indicates that no significant differences are noticed in any of the stability parameters for either study with the exception of a slight increase in [redacted] nm absorbance at the highest concentration of product [redacted], in Study 1. They state that no difference was noticed between storage at [redacted] Based upon both studies they request a stability of [redacted]

My review of the submitted data, including primary data, supports their observations regarding stability of the bulk. The addition of the clot-lysis assay (the more physiologically relevant assay), measurement of the two-chain form, and modified BM06.022, make Study 2 a more comprehensive and relevant measure of bulk stability. Over time, degradation of product would be expected to result in degradation products and modifications, an increase in the two chain form (increase in amidolytic activity, decrease in fibrinolytic and clot lysis activity), and increase in

aggregates and dimers. The specification for the parameters appear reasonable and are consistent with the specification for parameters that are in the formulated final product.

I note that there is a possible trend toward an increase in the—— modified form, however this is not definite. The specification for this parameter is defined as "no significant change over time." BM should quantitatively define how they determine what a significant increase is.

Studies included in the PLA and IND indicate that product from the · ——→ promoter are biochemically and pharmacokinetically equivalent, and that equivalent product is produced by the pilot and full scale manufacturing processes used in the stability analysis. Thus, BM indicates that Study 1 can support the currently produced product and their requested dating period of 24 months. BM should submit stability updates on the bulk lots G002 (48 months), G010, G011 (24 months), as soon as they are available (mid-January 96) to substantiate their proposed dating period. Since Study 2 provides the more relevant stability indicating parameters, this study should be used to definitively define the bulk dating period with supporting information from Study 1.

C.11.2. Test for Impurities

Several reports summarizing the impurities in bulk drug substance are described. Only results are presented, no primary data are provided. Assays used to measure these impurities are validated and appear in section C.10. or reference compendial methods (USP, CFR). The results are summarized:

Endotoxin testing by LAL of G002.00E, G009.00 to G018.00 batches detected 6.0 EU/ml or less. Retesting of lots with 6.0 EU/ml values were within specification of · ——

Pyrogen testing according to USP criteria using a 0.5 ml dose/kg that corresponds to 5-fold the single human dose was conducted on 4 batches. It is indicated that these batches met USP standards. It is stated that an additional 50 batches prior to the commercial production were tested as part of the LAL validation study (not submitted). LAL replaced pyrogen testing in the manufacture of commercial product as of batch G012.

Sterility testing according to CFR were performed on 11 batches and reported to be sterile. Using a USP approved method 20 of 21 fermentation batches contained only the production strain and no other microorganisms were detected. No mention is made of the organism(s) that contaminated the 21st batch.

Bacteriophage was not detected using a plaque assay in 24 batches.

Using an ELISA specific for host cell proteins, a maximum of 51 ppm and a mean and standard deviation (SD) of —— was detected in 11 batches.

Using an ELISA specific for [redacted], a maximum of 0.8 ppm with a mean and SD of 0.4 ± 0.2 ppm was detected in 11 batches.

Using [redacted], a maximum of 1.0% and a mean and SD of 0.8 ± 0.15% peak area corresponding to dimers was detected in 11 batches. No peaks corresponding to aggregates could be detected.

Using [redacted], a mean and SD of 0.8 ± 0.1, 1.3 ± 0.23 and 5.7 ± 1.31 was detected for the modified species [redacted] respectively, in 11 batches.

Nucleic acid (DNA) was measured using a [redacted] The maximum amount of DNA detected was 23 pg per 10 U reteplase dose in 11 batches.

From the results presented, levels of any impurity detected are reasonable and within specifications. All of these values fall within specification for the bulk.

C.11. Batch Analysis

This section contains batch analysis, test results and raw data for bulk drug on a pilot, 50g and 100g scale. The data are sufficiently labeled and of good quality for review. My review of these data indicate that overall the biochemical characterization of the bulk drug indicates a pure product with high quality, the amount of contaminants and altered reteplase molecules is minimal and relatively consistent between scales. The primary data support the results presented in the data summaries.

D.3. Composition of the Diluent

SWFI is provided by the contract manufacture [redacted] as an alternate. Representative certificates of analysis are included from both manufactures to indicate the SWFI pass USP requirements. A review of the certificate of analysis specifications based on USP requirements for SWFI indicated that they are acceptable. A reference to the master file for each manufacturer will have to be provided to establish the stability period for the SWFI.

D.4. Preservative

No preservative is used in reteplase

D.5. Final Containers and Closures

The components of the reteplase kit are listed (see table). The SWFI diluent, dispensing pin, syringe, from primary and alternative suppliers, and the needle supplier have supporting documentation in the PLA indicating that all these components can be used under the grand fathered regulatory clause, except for the dispensing pins that are cleared under 510(k).

Table 1
Reteplase Kit Components

Component	Composition	Manufacturer	Catalog Number
Drug Product	Reteplase 10 Unit vial	Boehringer Mannheim GmbH	NDC #53169-XXX-XX
Diluent	Sterile Water for Injection, USP in 10 mL glass vial	[redacted]	[redacted]
Alternate Diluent	Sterile Water for Injection, USP in 10 mL plastic vial	[redacted]	[redacted]
Syringe with needle	10 cc plastic syringe with 20 gauge 1 inch PrecisionGlide needle attached (sterile packed)	[redacted]	[redacted]
Vented dispensing pin	DP-1000 Mini-Spike® Dispensing Pin™ (sterile packed)	[redacted]	[redacted]
Alternate vented dispensing pin	EXACTA-MED® Dispensing Pin (sterile packed)	[redacted]	[redacted]
Needle	20 gauge 1 inch PrecisionGlide needle (sterile packed)	[redacted]	[redacted]
Kit	All above components	Boehringer Mannheim GmbH	NDC #53169-XXX-XX

Only approved packaging components that have met specifications will be used for the reteplase kit.

The composition of one vial includes 10 U reteplase as the bulk drug substance, 871.0 mg arginine, 268.6 mg phosphoric acid, and 1.0 mg polysorbate 20 as excipients. The actual content includes an additional [redacted] overfill to compensate for the non-withdrawable volume after reconstitution.

The container/closure system for reteplase consists of: 1) colorless 20 ml glass vial (type I borosilate glass; 2) rubber closure made of red chlorobutyl rubber, and; 3) aluminum cap. The glass and rubber components are stated as complying with the appropriate USP/Ph. Eur. monographs. A list of authorized suppliers and manufacturing specifications are supplied. Each component undergoes a testing procedure that includes general requirements, physical/technical tests, tolerances and relevant USP tests. In addition to these tests, the glass vials reference chemical tests, and the rubber closures, reference purity and identity tests, and a preparation list of reagents used in conducting these tests. A description of a sampling plan is provided that is based upon a statistical analysis of the batch size. Several compendial test methods are referenced from appropriate sources - USP/Ph.Eur, DAB, ISO, and DIN. Nominal values, tolerances and/or permissible limits are listed for component specifications and for contaminants.

D.6. Cleaning of Final Containers and Closures

The cleaning, sterilization, and treatment of the vials and rubber closures are described. The vials are washed using purified water with WFI as a final rinse, and automatically transferred through a depyrogenation tunnel into the filling room under laminar air flow. Summary reports of the validation of the vial washing machine, depyrogenation tunnel, and stopper washing/sterilizing machine are included. The complete validation studies are included in the ELA.

The validation study of the vial washing machine indicates that it is efficacious in removing glass particles during the wash cycle, and that washed vials are not contaminated with bioburden and endotoxin. The validation study of the depyrogenation tunnel indicates that in vials spiked with [redacted]

EU, respectively, remain per vial. Heat distribution, heat penetration, HEPA filter integrity test in heating, depyrogenation and cooling zone, and air velocity (as measured by particle counts) meet and generally exceed minimal specifications.

The stopper washing machine is designed to wash, rinse, siliconize and sterilize rubber stoppers. The rinsing is performed in purified water with the final rinse in WFI. The validation study using endotoxin - and bioburden - spiked stoppers indicate that it sterilizes, and removes endotoxin to the level of [redacted] or less and bioburden to a sufficient level.

E.1.A.1. Preparation of the Final Bulk Product

One batch of final product is defined as the load of the freeze dryer chamber - approximately [redacted] this can be as low as [redacted] vials due to normal loss during production.

The SOP for Shipping and Acceptance Testing and Release of Reteplase was reviewed (Report TQ 11-020-00). It is indicated that the Head of QC accepts/releases product. There is no acceptance testing performed for reteplase once it arrives at Mannheim. I have the following questions that by agreement with the ELA chair will be handled in the ELA review. Describe how the manufacturer ensures that shipping conditions are observed?

2. Raw Material Specifications

Specifications for various parameters including description, solubility, identity, purity, (including contaminants), microbiological examination, endotoxins and in-house testing procedures are provided for excipient material used in the formulation of reteplase 10 U - arginine ([redacted], concentrated phosphoric acid ([redacted]) and polysorbate 20 ([redacted]). In-house testing procedures describe tests for identity, description, purity, quality and content. Tests are performed for other contaminants as appropriate. Procedures and specifications for detecting chemical contaminants are also provided. These include 1) DNA ([redacted]) 2) endotoxin (LAL) - [redacted] (arginine, [redacted]; and microbiology - not more than [redacted] (arginine, polysorbate 20) and exclusion of certain organisms (*E. coli, P. aeruginosa, S. aureus* - absent in 1g, each), *Salmonellae* (absent in 10 g). Minimal instructions for storage are listed.

The listed product specification meet USP specifications for arginine, and polysorbate 20. The USP requirements for phosphoric acid indicate that ACS grade material should be used. Based upon my calculations of the added amounts of these components, and assuming the maximum allowable bioburden and endotoxin contamination, the amount of added contaminants per dose is minimal and pose negligible risk given the current manufacturing scheme that includes multiple filtration steps.

3. Description of the Manufacturing Process/In Process Specifications
The manufacturing process resulting in reteplase-lyophilizate 10 U is outlined in Figure D-6 below.

The manufacturer indicates that pivotal steps are carried out under aseptic conditions (typically class — in a class ~~ environment). The entire manufacturing process has been validated according to a pre-established Validation Master PLA (not provided). All equipment coming into contact with the drug substance, drug product, and excipient solution is sterilized before use.

The in process controls are performed at the various manufacturing steps as indicated (see table below).

Table. Overview of In-process Control Testing during Manufacture of Reteplase-Lyophilisate 10 U

IPC No.	Process step	Test parameter	Specification	Consequences if limits are exceeded
1	excipient solution (before sterile filtration)			
2	excipient solution (after single filtration)			
3	drug substance solution			
4	product solution (before first sterile filtration)			
5	product solution (after first sterile filtration)			
6	product solution (before second sterile filtration)			
7	product solution (after second sterile filtration)			
8	aseptic filling			
9	aseptic filling			
10	lyophilisate			

Legend: 1: investigation of cause and removal if applicable
2: consideration for performance and assessment of batch release control
3: consideration for calculation of manufacturing formula

The IPC seem reasonable for most process steps. However, the pooled bulk drug should be tested for sterility prior to mixing with the excipients as well as the final formulated bulk prior to filtration as part of the in-process controls. Limits should be set based upon the actual manufacturing experience. These tests are necessary to monitor sterility of the bulk during manipulation and to locate source of potential contamination in the process. For IPC #8, environmental monitoring, the "limits specified by the facilities" used to fill the commercial scale lots of reteplase should be checked during inspection.

The addition of potentially contaminated polysorbate to the formulated product solution prior to filtration does not seem to present a problem. The maximum calculated bioburden added from polysorbate based upon the maximum amount specified in the raw material, would only add The amount of contamination added from arginine would be CFU/ml in the prefiltered excipient mix. A sample of seven lots indicate that the bioburden of the excipient solution (arginine) prior to filtration ranges from 1-3 CFU/ml. Sterile filtration of the arginine mixture, and an IPC #2 with a bioburden of should sufficiently reduce contamination prior to addition to the unformulated bulk.

Based upon the maximum amount of endotoxin allowed in raw materials of endotoxin from arginine and rom polysorbate could be added to the formulated bulk. This would result in less than 5 IU of endotoxin per 10 U dose contributed from the excipients. However, the specification allows fo I after sterile filtration of the excipient solution and from the bulk specification for a total of at the aseptic filling stage. This results in a possibility of dose or ɔer 2 dose regimen that is within the CBER guidelines. If this EU limit in IPC #9 is exceeded, the stated consequences are 1) to investigate the cause and 2) consider the performance and assessment of batch release. There is no EU specification for the final vialed reteplase but it must pass the pyrogen test (CFR).

E.1.b. Sterilization

A procedure for sterilization is described. Validation summaries of solution preparation vessels, sterilization of utensils, validation of dry heat ovens and autoclave were reviewed. No apparent problems were noted. The full validations are described in the ELA.

E.1.c. Pooling of Bulk Drug

Up to batches of the bulk drug may be pooled for the manufacture of finished product. Since each batch has to meet product specifications that have reasonable limits on impurities and altered forms of reteplase, I do not foresee a problem with diluting substandard batches by pooling.

E.1.d. Lot Numbering System

A description of the lot numbers and the significance of every digit location in the

lot number is described. Each lot of inclusion bodies and bulk drug substance is also assigned a unique lot number. There is also internal batch numbering systems for fermentation and downstream production. The final product is assigned a unique lot number from which its entire history can be obtained. It appears from the description that the drug intermediates can be traced throughout the manufacturing process.

E.2. Filling the Final Product

A description of the filling of the final product is described. A filling machine, ——— is used to dispense reteplase. Rubber closures are provided by an automatic washer and steam sterilizer. All loading and transfer steps are conducted under class — aminar flow. A dedicated filling head is utilized to fill reteplase into sterile, depyrogenated . The — overfill is to allow for volume that is not withdrawn from the vial, to ensure that a full 10 ml dose can be administered. The relevant parts of the machine are cleaned as SIP/CIP. A validation summary is included in which the performance of the machine is validated. Validation tests were: media fill, microbiological challenge, SIP, challenge of pumps test, filling volume pumps, sterility of compressed air, test for viable particles and airborne particles count. All specifications for the validation were met. The machine was requalified after installation of additional components.

Since 10.8 mls equal to 10.8 Units of Reteplase are dispensed into each vial, does reconstitution with 10 mls of WFI as indicated in the labeling instructions provide an accurate 10 U dose of Reteplase? The labeling instructions indicate that all of the 10 mls injected may not be withdrawn. Is there any evidence to indicate that this variable amount of dose effects clinical efficacy? Will refer this to the clinical reviewer.

E.3. Methods to Insure Sterility

A description of the microbiological product/related studies are described. The bioburden in the excipient prior to filtration indicate that it low (1-3 CFU/ml), and within the range of the validated performance characteristics of the filter used to remove the bioburden from the excipient solution. The sterile filtration was validated on a scaled-down model system using *Pseudomonas diminuta* at the appropriate concentration. Results of three experiments indicate that reteplase does not interfere with the ability of the filter to produce a sterile reteplase solution. Sufficient information on the placebo solution and experimental set up to evaluate the results of the experiments is provided. A microbiological assessment of the reteplase holding period was determined by demonstrating that the reteplase fill solution did not support the growth of a wide range of bacteria, molds and yeasts when incubated at . The holding period was determined to be .

Container closure integrity, described in report CZ 7.2 (Vol 10, 140), was tested using a static ambient challenge test with vials in the upright and inverted position test, and a static immersion test immersing inverted vials in a solution of E. coli. These are relevant testing procedures for testing container closure and integrity.

Both tests described appropriate positive controls and were negative for organisms in the experimental reteplase containers. The tests were started in June of 1994 and will be completed in June of 1996 to cover the 2 year dating period. The latest test results for January 1995 indicate that the container/closure system is effective in preventing product contamination. The company must submit the container closure and integrity results when complete to substantiate the requested dating period.

E.4. Freeze Drying Procedures

After filling of the batch, the vials are lyophilized in an automated freeze dryer. The vials are loaded at C

After completion of the cycle, the vials are closed automatically and transferred to a capping station. Capped vials are inspected and tested for residual moisture using the Karl Fisher test.

E.4.b. Process Validation

The validation of the manufacturing process for final formulation and fill of reteplase 10 U on a commercial scale is described. The process validation is based upon the manufacturing process, a risk analysis to identifies critical processing steps, and validation experiments for these potentially critical process steps. The sponsor indicates that pilot scale validation was used to determine the limits for crucial process steps, and to establish parameters and ranges to allow for consistent manufacture. Subsequent commercial scale validation was to establish that consistancy of the manufacturing process that comply with the set specifications. Validation of the commercial scale process was demonstrated by means of manufacture and assessment of three MIX batches and the first commercial scale "full size verum" batch.

Pilot Scale Process Validation

The product is processed aseptically. Several sterile filtrations through a filter are performed at different points in the process with what appear to be appropriate IPC tests. There are several hold points in the manufacture that have been validated: freezing and thawing of the drug substance (lots), holding time of the thawed drug substance, holding time of the pooled drug substance, holding time of the excipient solution at stirring speed of the drug product solution, pH limits for the drug product solution, standing time of the mix after first filtration, finished filtered drug product during fill, precision of the filling devices, limits of the freeze dry process.

The validation studies measure biochemical, microbiological and IPC data relevant to the processing step. From the validation report, these process steps have been validated for hold times/conditions and process limits (see table).

Processing Step	Hold Times & Conditions/ Process Limits
1) freeze/thaw of drug substance (lots) -	
2) holding time of pooled (thawed) drug substance -	
3) holding time of excipient solution -	
4) holding time of reteplase drug product	
5) pH range	
6) stirring speed of drug product solution -	
7) sterile filtration	see below
8) filling precision	see below
9) lyophilization (limits)	see below

6) stirring at 3-5X faster did not affect product.
7) sterile filtration - bacterial challenge, IPC no protein loss for 4 batches; filter compatibility - worst case no protein loss (no protein adsorption), integrity tests meet IPC spec.
8) filling - IPC from 4 batches S.D. <1%, additional validation studies.
9) worst case approach and various experimental conditions on pilot batches to determine amount of variation allowed for freezing time, shelf temperature, primary and secondary drying.

Further validation information on lyophilization includes measurements of activity, biochemical data, residual moisture, and dissolution time. Results indicate that hours is sufficient for lyophilization leaving a safety window. Freezing time of batches indicate that freezing times from minutes comply with release specifications. Studies to assess the limits of primary drying temperatures and pressures were conducted, and acceptable deviations indicate that product quality is not lost if product temp doesn't exceed during the main drying. Secondary drying conditions are defined as up to Heat as high as +35°C did not result in any product changes.

Additionally, a survey of residual moisture indicated that for several batches moisture was or less. As a result of this study and a request from CBER, this specification was lowered from for commercial scale production. No effect of residual moisture from stoppers was observed on product parameters. No adsorption of protein to filling devices was detected as supported by data of process control samples for four batches. There is a statement indicating that the tubing is compatible with the drug product although no data are provided.

The validation data provided indicate that the product is stable for the latest time

point in the stability study. However, validation times and process limits are based upon the last available time point performed in the stability study for intermediates 1, 2, 3, 4, and 5. These stability studies need to be conducted for an additional amount of time beyond the last time point in order to use the currently defined hold times. Alternatively, the hold times and process limits could be shortened to an appropriate time less than the maximum time point for which there are valid data.

Commercial Scale Process Validation

Validation batches were manufactured in the facility and with the equipment intended for commercial production. As described and discussed with the FDA at the pre-PLA meeting on December 13, 1994, these three batches were manufactured as [redacted]

Each [redacted] batch was assessed for thawing and mixing of the bulk, protein absorption to filters and the filling system, and lyophilization. Mostly IPC parameters with some additional biochemical results were compared with release specifications. Biochemical data indicate that no significant change was noted with the product during any manufacturing step tested. All process parameters and IPC data were found to comply with the specifications for all three validation batches. Activity and biochemical data were within specification for the validation batch samples at various hold times throughout the process.

Although variations in the drying were assessed in the pilot validation experiments, since lyophilization changed between pilot and commercial scale, additional studies were conducted on commercial scale product. For lyophilization an additional test, the temperature difference, was used for two batches instead of the pressure-drop test to determine the primary end of drying. Note that during the validation of the freeze drying process since the dryer runs have preset, fixed parameters (except for the last drying) the upper and lower ranges could not be tested. All product specifications meet IPC, and lot release specifications, including the new residual moisture content limit of [redacted] The variations in the three validation batches are within the range of the dryer.

From these [redacted] commercial scale batches, typical processing times for steps are, an overnight hold time of [redacted] and with the other steps a total process time of [redacted] was determined. These process times are within the validated times. From the confirmatory batch the process steps are indicated to have taken (hr:min) [redacted] Biochemical data is presented

to indicate that product at various stages (indicated as [redacted] throughout the process does not result in a degradation in product quality. The individual parameters for time limits between processing steps [redacted] for specific product steps do not have limits. While the overall processing time and some identified steps would be supported by the validation data from the pilot study, the time limits of the individual steps need to be defined (preparation of product solution, thawing time, solution preparation, storage of product solution overnight, and filling process).

There are slight changes in the manufacturing practice between the pilot and commercial scale. These include elimination of the refreezing of pooled drug substance, mixing of arginine and polysorbate prior to, instead of with, drug substance, and slighly different lyophilization conditions. The composition of the formulation, thawing of drug substance, sterile filtration, and filling are unchanged. Thus the the pilot scale data can support the consistancy of the commercial scale process at several steps.

Process Validation - Confirmatory Batch Commercial Scale
Confirmatory process validation results were obtained during manufacture of the first and most recent commercial scale "full size verum" batch (batch size [redacted] approach). The batch complies with all IPC and product release specifications.

Microbiological Process Validation
Microbiological process validation comprised studies on bioburden, sterile filtration, and holding periods for the drug product solution, as well as media fills with the actual commercial scale equipment. These were used in-part to validate conditions and intermediate hold times.

E.5. Reprocessing
Reprocessing of final fill reteplase is not allowed.

Other sections related to assigned review sections

Final Product Stability
The final vialed reteplase stability data was reviewed. BM claims appear valid after reviewing the data. The stressed data of final vialed reteplase at 40°C do not indicate any degradation for parameters measured after 12 months of storage. BM concludes that the manufacturing process is validated within the limits of the process parameters. My review of these data support their conclusion

Final Product Dating - The date of manufacture needs to be defined as the first sterile filtration of the final formulated bulk so as to conform to 21 CFR 610.50. For any filled lots intended for distribution, the difference between the current date of manufacture and the redefined date as indicated above needs to be provided.

Batch Records. As part of reviewing certain sections I reviewed the manufacturing instructions/batch records submitted in the PLA for production of the bulk and

substance. In several areas in both manufacturing sites the batch records are deficient in information or correct presentation. This has generated the questions listed below.

Questions

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Items for review on inspection: